

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number  
**WO 01/68226 A2**

(51) International Patent Classification<sup>2</sup>: **B01D 57/02, C12Q 1/24, C12N 5/00, A01K 67/02 // A61K 35/52**

(21) International Application Number: **PCT/GB01/00982**

(22) International Filing Date: 8 March 2001 (08.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0006051.7 14 March 2000 (14.03.2000) GB

(71) Applicant (for all designated States except US): **THE UNIVERSITY OF BRISTOL [GB/GB]; Senate House, 4th Floor, Tyndall Avenue, Bristol BS8 1TH (GB).**

(72) Inventor; and

(75) Inventor/Applicant (for US only): **CUMMING, Ian [GB/GB]; The Mews Building, Division of Animal Health & Husbandry, Langford House, Langford, Bristol BS40 5DU (GB).**

(74) Agent: **HEATON, Joanne, Marie; Stevens Hewlett & Perkins, 1 St. Augustine's Place, Bristol BS1 4UD (GB).**

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

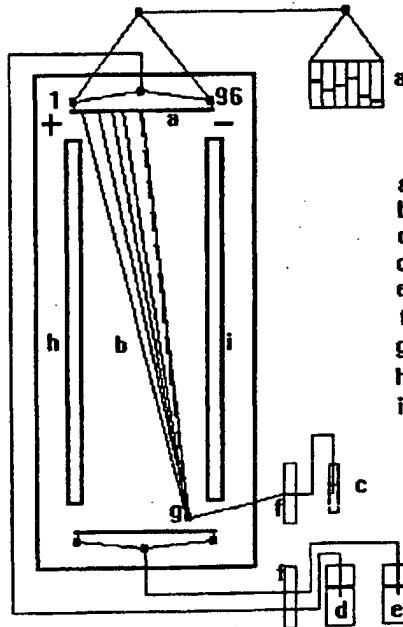
(84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).**

**Published:**

— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: A METHOD OF SORTING CELLS



**a = fraction collection.**

**b = separation area.**

**c = sample.**

**d = counterflow medium.**

**e = separation medium.**

**f = fluid pumps.**

**g = sample injection port.**

**h = anode electrode.**

**i = cathode electrode.**

**WO 01/68226 A2**

(57) Abstract: A method of sorting cells, particularly, but not exclusively, sperm cells, is described. The method comprises the use of a surface decontamination medium to strip the cells of any extra-cellular surface contamination and to then subject to stripped cells to a charge based separation.

**CLAIMS**

1. A method of sorting cells, the method comprising the steps of treating the cells to remove extra-cellular surface contamination and subjecting the treated cells to a charge-based separation process.
2. A method according to claim 1, in which endogenous cell membrane properties are maintained during the removal of surface contamination.
3. A method according to claim 1 or claim 2, in which surface contamination is removed from the cells using a medium containing egg yolk or derivatives thereof.
4. A method according to claim 3, in which the egg yolk or derivative thereof is present in the surface contamination removal medium at a concentration of up to 30% volume/volume.
5. A method according to any claim 3 or claim 4, in which the egg yolk or derivative thereof comprises chicken egg yolk.
6. A method according to any preceding claim, in which the charge based separation process is an electrophoretic process.
7. A method according to claim 6, in which the electrophoretic process is conducted in a mobile liquid separation medium.
8. A method according to claim 6 or claim 7, in which the electrophoretic conditions of the electrophoretic process are constant throughout the separation chamber.

9. A method according to claim 7 or claim 8, in which the electrophoretic process comprises free-flow electrophoresis.
10. A method according to any preceding claim, in which the sorted cells are selected from the extreme poles of the separation range.
11. A method according to claim 10, in which the sorted cells are selected from both extreme poles of the separation range.
12. A method according to any preceding claim in which the cells are sperm cells.
13. A method according to claim 12, in which the surface contamination removed comprises accessory proteins, secretions added to the sperm cells during the process of cell membrane assembly, secretions added to the sperm cells during the process of cell maturation, secretions added to the sperm cells during the process of cell transport, secretions added to the sperm cells during the process of cell storage within the male reproductive tract, micro-organisms from the male reproductive tract and other contaminants or micro-organisms introduced during ejaculation.
14. A method according to claim 12 or claim 13, in which the cells are mammalian sperm cells.
15. A method according to any one of claims 12 to 14, in which the sorted cells are sperm cells bearing X-chromosomes.
16. Sperm cells bearing X-chromosomes sorted using a method according to any preceding claim.

17. A method of producing non-human female embryos, the method comprising the steps of treating sperm cells to remove extra-cellular surface contamination, subjecting the treated sperm cells to a charge-based separation process to separate the X- and Y- chromosome bearing sperm cells, and using X- chromosome bearing sperm cells separated thereby to fertilise an egg.
18. A non-human female embryo produced using the method of claim 17.